IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Lee et al.	§	ART UNIT:
	§	1647
FILED: November 16, 2000	§	
	§	EXAMINER:
SERIAL NO.: 09/714,692	§	Bunner, B.
	§	
FOR: Method of Inhibiting Angiogenesis By Administration of A Corticotropin Releasing Factor Receptor 2 Agonist	§	DOCKET: D6233CIP

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Alexandria, VA 22313

APPEAL BRIEF

This Brief is in furtherance of the Notice of Appeal filed in this case on September 9, 2004. The fees required under 37 C.F.R. §41.20(b)(2) and any other required fees are dealt with in the accompanying TRANSMITTAL OF APPEAL BRIEF.

Date: Oct 15, 2004

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INDEX OF SUBJECT MATTER

I. REAL PARTY IN INTEREST

The real party in interest is Research Development Foundation, the Assignee, as evidenced by an Assignment recorded in the Patent and Trademark Office at Reel 011466, Frame 0830 on January 26, 2001.

II. RELATED APPEALS AND INTERFERENCES

Appellant is aware of no other appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

Originally claims 1-27 were filed with this Application. Claims 1-19 and 24-27 were canceled. The pending claims 20-23 are being appealed of which claim 20 is an independent claim.

IV. STATUS OF AMENDMENTS

Subsequent to the final rejection mailed March 4, 2004, Applicants submitted a Response After Final which canceled claims 1-19 and 24-27. In an Advisory Action mailed August 19, 2004, the examiner stated that the amendment will be entered for purpose of appeal. All pending claims are shown in Appendix A.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Claims 20-23 are directed to a method of using a CRFR2 agonist such as urocortin or corticotropin releasing factor to inhibit angiogenesis in a target tissue such as heart, brain, pituitary, gonad, kidney, adipose, or the gastrointestinal tract. This method of the present invention is applicable to an individual having cancer or diabetic retinopathy.

The present invention provides data that indicate Corticotropin Releasing Factor Receptor 2 (CRFR2) null mutant mice exhibit an increase in the size and number of blood vessels in

various tissues. Figure 7 shows an increase in number and size of blood vessels in the anterior pituitary (Figure 7B), white adipose tissue (Figure 7D) and dorsal brain surface (Figure 7F) in corticotropin releasing factor receptor 2 null mutant mice. Microfil perfused tissues also indicate increased vessel size and number in dorsal brain surface (Figure 9A), large intestine (Figure 9B) and heart (Figure 9C). The major vessels in kidney, adrenal glands and testis are significantly increased in size in corticotropin releasing factor receptor 2 null mutant mice relative to those of control mice (Figure 10). Since corticotropin releasing factor receptor 2 and its activity have been localized within the endothelial cell layer of blood vessels, the data presented herein indicate that corticotropin releasing factor receptor 2 plays a significant role in regulating angiogenesis (page 48, lines 20-21; page 49, line 4). In view of the data disclosed herein, one of ordinary skill in the art would conclude that well-known corticotropin releasing factor receptor 2 agonists such as urocortin and CRF could be used to inhibit angiogenesis.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Claims 20-23 stand rejected under 35 U.S.C. §102(b) as being anticipated by **Villalona-Calero** et al.

VI. ARGUMENT

Rejection Under 35 U.S.C. §102

Claims 20-23 stand rejected under 35 U.S.C. §102(b) as anticipated by **Villalona-Calero** et al. The Examiner contends that **Villalona-Calero** et al. teach administering human corticotropin releasing factor to the same subject population and the same tissue as recited in Applicants' claims, therefore human corticotropin releasing factor inherently possesses angiogenesis-inhibiting activity as claimed herein. For the reasons outlined *infra*, Applicants respectfully request that this rejection be withdrawn.

The present invention is drawn to a method of using a corticotropin releasing factor receptor 2 (CRFR2) agonist to inhibit

angiogenesis in a target tissue. In contrast, Villalona-Calero et al. teach a method of using human corticotropin releasing factor (hCRF) to treat patients having peritumoral brain edema. Villalona-Calero et al. teach human corticotropin releasing factor inhibits vascular leakage of plasma constituents in response to injury (last sentence on page 71). Villalona-Calero et al., however, do not teach or suggest a method of using corticotropin releasing factor to inhibit angiogenesis in a target tissue as claimed by the Applicants.

Angiogenesis is a process of forming new blood vessels. Villalona-Calero et al. do not teach or suggest any scientific relationship between angiogenesis and the prevention of vascular leakage, and one of ordinary skill in the art would readily recognize that these are two distinct biological processes. Absent a teaching that shows any relationship between angiogenesis and anti-edematous effects, one of ordinary skill in the art would have no reasonable and logical scientific basis to recognize or suspect that human corticotropin releasing factor inherently possesses angiogenesis-inhibiting activity. The Examiner has not produced any scientific evidence or legal argument to the contrary.

The Examiner rejects the Applicants' pending claims solely on the basis that the prior art taught using the same type of biomolecule in the same subject population and the same tissue as claimed herein. The Examiner's assertion that human corticotropin releasing factor inherently possesses angiogenesis-inhibiting activity is not supported or suggested by any scientific reasoning or data. According to the Examiner's reasoning, patenting of any new or novel method of using a compound is precluded once the prior art has described using the compound for whatever purpose in similar target tissues. Applicant submits that such rejection is overtly broad and without a legal basis.

Applicant submits that the Examiner has failed to provide scientific rationale or legal evidence demonstrating inherency. In relying upon the theory of inherency, the Examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art. *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990). The fact that a certain result or characteristic may occur or be present in the prior

art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993), *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). To establish inherency, the extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient. *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999).

Accordingly, Applicant submits that the Examiner has not provided any basis in fact and/or technical reasoning to reasonably support the legal determination that the allegedly inherent characteristic of inhibiting angiogenesis necessarily flows from the teaching of Villalona-Calero et al. The Examiner has not provided any extrinsic evidence that clearly shows the allegedly inherent characteristic of inhibiting angiogenesis is necessarily present in Villalona-Calero et al. and that it would be so recognized by

persons of ordinary skill absent disclosure presented in the instant application.

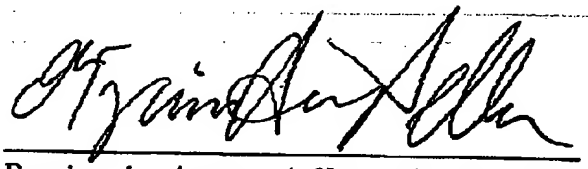
Applicants respectfully submit that the Examiner has not established that **Villalona-Calero** et al. anticipates the critical elements of Applicants' claim. As always, "[a]nalysis begins with a key legal question – what is the invention claimed?" since "[c]laim interpretation ... will normally control the remainder of the decisional process," Panduit Corp. v. Dennison Mfg. Co., 810 F.2d 1561, 1567-68, 1 USPQ2d 1593, 1597 (Fed. Cir.), cert. Denied, 481 U.S. 1052 (1987).

To meet the requirements of the claims on appeal, the corticotropin releasing factor receptor 2 agonist must inhibit angiogenesis in the tissue. As discussed *supra*, the Examiner has not provided any scientific evidence that **Villalona-Calero** et al. demonstrates this requirement of Applicants' claim.

For the reasons given above, Applicants respectfully urge that the decision of the Examiner should be reversed, and that claims 20-23 be allowed.

Respectfully submitted,

Date: Oct 15, 2005



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CLAIMS APPENDIX

20. (previouslypresented) A method of inhibiting angiogenesis in a target tissue comprising the step of administering a Corticotropin Releasing Factor Receptor 2 (CRFR2) agonist to said target tissue, wherein said CRFR2 agonist inhibits angiogenesis in said tissue.

21. (previouslypresented) The method of claim 20 wherein said CRFR2 agonist is selected from the group consisting of urocortin and corticotropin releasing factor.

22. (original) The method of claim 20, wherein said tissue is selected from the group consisting of heart, brain, pituitary, gonad, kidney, adipose, and gastrointestinal tract tissues.

23. (original) The method of claim 20 wherein said angiogenesis is inhibited in an individual having a pathophysiological condition selected from the group consisting of cancer and diabetic retinopathy.

EVIDENCE APPENDIX

Villalona-Calero et al., Ann. Oncol. 9: 71-77 (1998).

Annals of Oncology 9: 71–77, 1998.

Original article

A phase I trial of human corticotropin-releasing factor (hCRF) in patients with peritumoral brain edema

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Summary

Background: Human corticotropin-releasing factor (hCRF) is an endogenous peptide responsible for the secretion and synthesis of corticosteroids. In animal models of peritumoral brain edema, hCRF has significant anti-edematous action. This effect, which appears to be independent of the release of adrenal steroids, appears mediated by a direct effect on endothelial cells. We conducted a feasibility and phase I study with hCRF given by continuous infusion to patients with brain metastasis.

Patients and methods: Peritumoral brain edema documented by MRI and the use of either no steroids or stable steroid doses for more than a week were required. MRIs were repeated at completion of infusion and estimations by dual echo-image sequence (Proton density and T2-weighted images) of the amount of peritumoral edema were performed. The study was performed in two stages. In the feasibility part, patients were randomized to receive either 0.66 or 1 μg/kg/h of hCRF or placebo over 24 hours. The second part was a dose finding study of hCRF over 72 hours at escalating doses.

Results: Seventeen patients were enrolled; only one was receiving steroids (stable doses) at study entrance; dose-limiting toxicity (hypotension) was observed at 4 μg/kg/h × 72 hours in two out of four patients, while zero of five patients treated at 2 μg/kg/h developed dose-limiting toxicities. Flushing and hot flashes were also observed. Improvement of neurological symptoms and/or exam were seen in 10 patients. Only small changes were detected by MRI. Improvement in symptoms did not correlate with changes in cortisol levels, and changes in cortisol levels were not correlated with changes in peritumoral edema.

Conclusions: hCRF is well tolerated in doses up to 2 μg/kg/h by continuous infusion × 72 hours. Hypotension limits administration of higher doses. The observation of clinical benefit in the absence of corticosteroids suggests hCRF may be an alternative to steroids for the treatment of patients with peritumoral brain edema. Further exploration of this agent in efficacy studies is warranted.

Key words: brain edema, corticotropin-releasing factor, hCRF, proton density, T2-weighted

Introduction

Peritumoral brain edema is a significant cause of morbidity and mortality. Few advances in this field have been made since the introduction of steroids more than 30 years ago. Substantial *in vivo* data suggest that administration of human corticotropin-releasing factor (hCRF) could be beneficial in the management of this complication. We report a phase I trial of hCRF administered to patients with peritumoral brain edema.

CRF, a naturally occurring 41-residue neuropeptide, was first isolated from sheep hypothalamic extracts in 1981 [1]. Its structure is highly conserved among species; the molecules in humans and rats are identical and differ by seven amino acids from the ovine sequence and by two from the porcine structure [2, 3]. CRF is found primarily in the hypothalamic paraventricular nucleus, but it has also been identified in cerebral cortical interneurons, the limbic system, brain stem and spinal cord [4]. CRF is the predominant regulator of adreno-corticotropic-hormone (ACTH) formation and release by the pituitary. It causes a dose-related increase in synthesis and secretion of ACTH, lipotropin, β-endorphin and N-proopiomelanocortin from the pituitary gland. This peptide binds to specific high-affinity receptors on the pituitary cell surface and through the intracellular messengers calcium and cyclic AMP initiates a cascade resulting in the release of the above hormones [4].

Exogenous CRF has been administered extensively to patients in pharmacological doses (bolus doses of 1–5 μg/kg; and continuous infusions of 2,000 μg/24 hours), in studies of endocrine function, without significant side effects [5]. Facial flushing and mild dyspnea have been observed at these doses, while higher doses (up to 30 μg/kg) have been associated with hypotension, tachycardia, arrhythmias and mental 'absences' [6].

hCRF inhibits vascular leakage of plasma constituents in response to injury and to the injection of vaso-

active compounds [7, 8]. Using an experimental model of vasogenic peritumoral brain edema, Tjuvajev et al. observed a dose-dependent decrease (calculated ED_{50} = 59 μg/kg BID) in vasogenic peritumoral brain edema as assessed by proton density weighted (PDW) and T1 weighted (T1W) MRIs in rats after subcutaneous (s.c.) administration of hCRF [9]. Intracerebral tumors were produced in this study by intracerebral inoculation of the RG2 rat glioma cell line. To compare the effects of hCRF and dexamethasone on the permeability of the blood brain barrier (BBB) and on water content of tumor and peritumoral brain tissue, rats bearing RG2 tumors were divided into three groups of 18 rats. Each group received treatment for three days, starting on day 7 after tumor inoculation, with either hCRF (100 μg/kg s.c. BID), dexamethasone (1 mg/kg i.m. BID), or saline (0.1 ml i.m. BID). MRIs were obtained immediately before treatment, and immediately after treatment had ended. Reduction of contrast enhancement and of proton density signal on MRI was seen on the tumor and peritumoral brain in the hCRF and dexamethasone treatment groups, in contrast to an increase on the above parameters in the saline treated animals. Direct measurements of tissue water content and peritumoral brain confirmed the radiological findings. The average survival time was significantly prolonged for the hCRF-treated animals bearing intracerebral tumors (35 days), compared to the dexamethasone-treated rats (28 days, $P < 0.05$) and to the saline-treated control group (22 days, $P < 0.0001$). hCRF was also able to decrease edema in adrenalectomized rats, suggesting its action does not depend on the release of corticosteroids.

Patients and methods

Patient selection

Patients were selected on the basis of the following eligibility criteria: (a) patients with primary or secondary brain tumors with evidence of evaluable edema on CT-Scan; (b) at least 18 years of age; (c) performance status of 50% or higher on the Karnofsky scale; (d) stable steroid dose over the previous week or no prior steroids; (e) ability to undergo two MRIs; (f) no significant cardiac, pulmonary or cerebrovascular (other than brain tumor) disease; (g) no brain abscess or other infectious process; and (h) no concomitant chemotherapy or radiation therapy. Patients severely symptomatic from cerebral edema requiring immediate treatment with steroids, mannitol or hyperventilation were considered not eligible for the study.

Dosage and drug administration

Immediately after obtaining an MRI, hCRF was given to patients intravenously, by continuous infusion (CI). In the first part of the study eight patients were randomized to receive either hCRF at 0.66 μg/kg/h, or hCRF at 1 μg/kg/h, or placebo, over 24 hours. Investigators and patients were blinded to the identity of the study medication. Both 0.66 μg/kg/h (i.e., 950 μg/24 hours for a 60 kg subject) and 1 μg/kg/h (1440 mcg/24 hours) were considered safe starting doses in view that doses up to 2,000 μg of CRF have been administered to human subjects by continuous infusion over 24 hours with minimal side effects [5, 6]. The placebo arm was included with the purpose of controlling for side effects. Toxicities were graded according to the National Cancer Institute (NCI) toxicity criteria [10]. In the event of no dose limiting toxicities (DLT) in the first part of the study, the duration of hCRF infusion was increased to 72 hours in the second part of the study. Given its dose finding purpose, no placebo arm was included in the second part of the trial. The dose of hCRF was doubled until DLT appeared. The maximum-tolerated dose (MTD) was defined as the highest dose at which no more than one of six new patients (or none of five) developed DLT. DLT was defined as at least one of the following: (1) an ANC less than 500/μl for longer than 5 days, or associated with fever; (2) a platelet count less than 25,000/μl (3) nonhematologic toxicity = NCI grade 3, except for nausea and vomiting despite treatment with an aggressive (serotonin receptor antagonist-containing) antiemetic regimen; and (4) NCI grade 4 vomiting despite treatment with an aggressive antiemetic regimen. hCRF was provided by Neurobiological Technologies INC, Richmond CA, in vials containing a lyophilized mixture of 0.2 mg of hCRF and 10 mg of mannitol. Sterile vials containing 10 mg of lyophilized acidified mannitol were used as placebo. Two ml of sterile 0.9% sodium chloride were used to reconstitute hCRF or placebo. The calculated volume of test article or placebo was added to 250 ml of sterile 0.9% sodium chloride solution (USP), and shaken gently. The 250 ml solution was infused intravenously at approximately 20.8 ml/hour over 12 hours by peristaltic pump. At 12 hours, and every 12 hours for 72 hours infusions, the infusion bag was replaced with another prepared in the same manner. Since no preservative was used, the solution was administered immediately after preparation.

Patient assessment and laboratory data

Vital signs were obtained at 30; 15; and one minute before hCRF or placebo administration; every five minutes for 30 minutes from the start of the infusion; every 15 minutes for 30 additional minutes; every 30 minutes for 2.5 hours; every 60 minutes for the next four hours; and every 12 hours thereafter. Neurological assessments as per the modified Mathew's scale [11] were determined at baseline and every 12 hours until the infusion finished. In addition, for the patients receiving 24 hours hCRF or placebo infusions, neurological assessments by the modified Mathew's scale were also performed every four hours during the first 12 hours of infusion. Cortisol and ACTH levels were determined in the patients receiving 24-hour infusions at: pre-drug administration; 90 minutes; eight hours; 12 hours after starting the infusion; and immediately after stopping the drug. For the patients receiving 72-hour infusions, Cortisol and ACTH were measured at: baseline; at 60 minutes and 12 hours after starting the infusion; and immediately after stopping the drug.

MRI assessment of peritumoral edema

MRIs (GE Signa 1.5 Tesla with head restraining system) were obtained immediately before starting and repeated at the completion of the infusion. Each study consisted of axial MR images of the brain acquired using a spin-echo pulse sequence with relaxation time (TR) = 2400 ms, first echo time (TE1) = 16 ms and second echo time (TE2) = 80 ms (Figure 1A). The early echo produced a proton density weighted (PDW) image (Figure 1B), while the late echo produced a T2 weighted (T2W) image of the same slice of brain tissue (Figure 1C). Each slice was 5 mm thick with a 1.5 mm interslice gap. The image was a 256 x 256 matrix with a 240 mm field of view. Axial T1 weighted images (TR = 600 ms, TE = 10 ms) with contrast agent (Gd-DTPA) were used to define the tumor border. Edema-like components were separated from other tissues in MR images using a two-feature segmentation scheme [12–16]. The features were PDW and T2W values from the early and late echo images, respectively. A 2-D histogram was created in which the X-axis was the PDW value and the Y-axis was the T2W value. A typical histogram is shown as an image in Figure 2. Since edema-like regions have distinct PDW and T2W values, they can be readily identified as a unique cluster of points in the histogram. In the histogram in Figure 2, a region of interest (ROI) was placed around this cluster. ROIs were

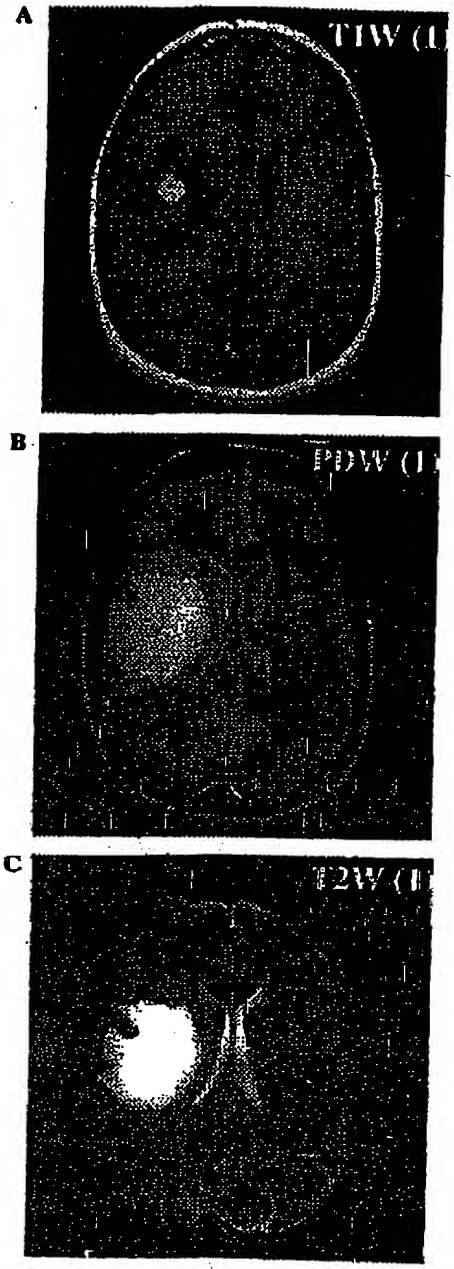

Figure 1. MRI scans used to evaluate degree of edema in each patients; (A) typical axial MRI T1 weighted (T1W) image: the light area is the tumor and the slightly darker area around it is the edema; (B) proton density weighted (PDW) image; (C) T2 weighted (T2W) image.

traced in the histogram for each slice where edema clusters were seen. Software was written that automatically segments the edema in the original images given the user-defined ROIs in the histogram for each slice. The edema volume was then calculated by multiplying the number of segmented voxels (*smallest units in a three-dimensional image*) by the voxel size. Some segmentation errors occurred due to misclassification. To compensate for this, pixels (smallest units in a two-dimensional image) in the segmented image that were not connected to the major edema were discarded before the volumes were calculated. The reproducibility of this method of tissue segmentation has a < 3% coefficient of variation [17].

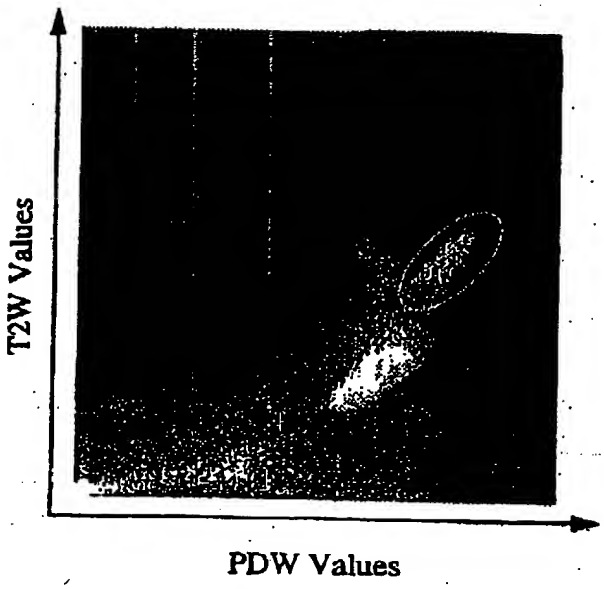

Figure 2. Segmentation of edema from other tissues and waterlike components. A two-dimensional histogram was created using the PDW as the X-axis and the T2W as the Y-axis. Edema is represented by the bright cluster of points outlined in white.

Data analysis

Relevant parameters calculated from the data included: 1) percentage changes from baseline on MRI measurements of water content; and 2) percentage changes from baseline on serum cortisol levels at 60–90 min; at 12 hours; and at the end of hCRF infusion. The above parameters were calculated using the following formula:

$$\%\ change = \frac{100 \times (\text{post-treatment value} - \text{pre-treatment value})}{\text{pre-treatment value}}$$

The Wilcoxon Rank Sums test was used to determine if symptomatic improvement correlated individually with % change from baseline in serum cortisol at 60–90 min; at 12 hours; and at the end of hCRF infusion, or if symptomatic improvement correlated with % change from baseline on MRI measures of water content. Univariate correlation analysis was performed to examine the relationship between changes (% from baseline) in peritumoral edema by MRI and percentage changes in serum cortisol levels at 60–90 min; at 12 hours; or at the end of hCRF infusion. Statistical analysis was performed using the JMP version 3.1 statistical software program (SAS Institute, Cary, NC).

Results

Seventeen patients were enrolled: nine males; eight females; median age 53. All patients had newly diagnosed metastatic disease to the brain from the following primaries: lung (six), breast (five), renal (three), unknown primary (two) and lymphoma (one). Only one patient had been receiving steroid treatment (stable doses), the rest had not received any treatment for their brain metastasis. In the first part of the study three patients received 0.66 μg/kg/h of hCRF over 24 hours, three patients received 1 μg/kg/h of the drug over 24 hours, and two patients received placebo. In the second part of the study, the hCRF infusion was given over 72 hours. The dose of hCRF was first escalated to 2 μg/kg/h, and

Table 1. Number of patients with toxicity (NCI grade).

hCRF dose (mcg/kg/hr)	No. of pts	Duration (hrs)	Hypotension	Facial flushing	Diarrhea	SOB	Nausea	Pain	Edem.
0	2	24	0	0	0	0	0	0	0
0.66	3	24	0	0	I (II)	0	I (II)	0	0
1.00	3	24	0	0	0	0	0	0	0
2.00	5	72	I (I)	3 (I, II)	0	I (II)	0	0	0
4.00	4	72	2 (III)	4 (II)	0	0	I (II)	I (II)	I (II)

Abbreviation: SOB – shortness of breath.

Table 2. Symptomatology improvement with hCRF.

Subject	Dose (mcg/kg/hr)	Site of tumor	Symptom	Physical finding
1	0.66	Cerebral hemispheres (multiple)		Improvement in muscle strength i all extremities
4	0.66	Lt temporal, inferior Lt frontal, superior Rt frontal	Resolution of headache	Resolution of focal seizure activit Resolution of ataxic gait
5	1.00	Rt parietal lobe		Improvement in strength of left arm flexors
6	1.00	Rt frontal lobe, head of caudate, cortico-medullary junction		Improvement in facial weakness, muscle strength Rt lower extrem- *ity, and normalization of reflexes*
9	1.00	Rt parietal lobe with mass effect and midline shift to the left	Resolution of headache, dizziness, and pressure sensation in back of neck	
10	2.00	Rt cerebellar hemisphere with compression of 4th ventricle		Improvement in muscle strength Lt upper and Lt lower extremity
12	2.00	Lt parietal lobe		Resolution in twitching on Rt ey lid. Resolution of conjugate eye deviation
14	4.00	Inf Rt cerebellum, Lt inf cerebellar peduncle, with mass effect on 4th ventricle Lt mesencephalon to thalamus		Improvement in Rt upper and lower extremity dyscoordination
15	4.00	Rt occipital, Lt occipital, Rt posterior parietal	Improvement in short-term memory loss	
17	2.00	Lt temporal lobe with mild Lt to Rt shift, Rt temporal lobe		Improvement in headache

later to 4 μg/kg/h, when no dose-limiting toxicities were observed in three patients at 2 μg/kg/h. Dose-limiting toxicities were observed in two out of four patients treated at 4 μg/kg/h. Further expansion of the 2 μg/kg/h dose to a total of five patients failed to reveal dose-limiting toxicities.

Toxicities

Table I summarizes the toxicities observed. No toxicities were observed in the patients receiving placebo. Grade II diarrhea, and grade I nausea, in one patient (0.66 μg/kg/h) were the only toxicities observed in the 24-hour hCRF-treated group. At the 2 μg/kg/h/72 hours dose level, facial flushing was seen in three out of five patients. Transient, mild hypotension (BP 96/34), which resolved without interruption of the drug, was seen in one patient. Epigastric burning (one patient), lethargy (one patien and mild shortness of breath (one patient) were al observed. Of interest was the observation that tw patients with a significant elevation in blood pressure study entry became normotensive during the infusio The 4 μg/kg/h/72 hours dose level was poorly tolerat with two out of four patients developing grade III hyp tension during the first day of infusion. Rechallenge wi the drug, performed in one patient, resulted again hypotension. All four patients developed facial flushin extending to trunk and extremities in two of them. Pe orbital and lower extremity edema in one patient, nas congestion in one patient, and grade II nausea a vomiting in another patient, were also observed. Pain the right neck and jaw with radiation to the tempo area accompanied the hypotension in one of the p tients.

Table 3. Mathew's scale scores of patients on hCRF.

Patient	Dose	Baseline	4 h	8 h	12 h	24 h	36 h	48 h	60 h	72 h
1	0.66	77	85	78	78	78	–	–	–	–
2	0.00	85	85	85	85	85	–	–	–	–
3	0.66	85	85	85	85	85	–	–	–	–
4	1.00	85	84	85	85	85	–	–	–	–
5	1.00	76	76	76	69	70	–	–	–	–
6	1.00	63	75	73	73	72	–	–	–	–
7	1.00	85		85	85	85	–	–	–	–
8	0.00	78	78	78	–	78	–	–	–	–
9	2.00	100			89	88	67	88	88	90
11	2.00	100	–	–	100	100	100	100	100	100
12	2.00	98	–	–	99	99	99	99	97	99
13*	4.00	100	–	–	–	–	–	–	–	–
14	4.00	100			100	100	100	100	100	100
15	4.00	100	–	–	100	100	100	100	100	100
16*	4.00	100	–	–	78	–	89	–	–	–
17	2.00	100	–	–	100	100	100	92	100	100

* Treatment discontinued due to hypotension.

Effect on edema and cortisol levels

Table 2 illustrates the clinical benefits observed in patients receiving hCRF. Ten of the 15 patients who received hCRF had some improvement in neurological symptoms or physical findings. In addition, another patient who had lymphoma-related pruritus and long-standing rash resistant to steroids, experienced resolution of both ailments while receiving hCRF. Two patients were symptom free and had no neurological findings pre- and post-infusion. Two patients had their infusion discontinued early due to the appearance of hypotension, and one patient experienced some worsening of his symptoms. No improvement on physical exam or in symptoms was seen in the patients receiving the placebo. Mathew's scale scores are depicted on Table 3. No significant trends in any particular direction were detected by this method of neurological assessment. MRI measurements of tumor and peritumoral water content pre- and post-infusion are illustrated in Figure 3. Among 14 patients evaluable by MRI, eight had a decreased in edema (including one placebo-treated patient), while six had their edema increased. Table 4 illustrates the cortisol/ACTH levels at different times during the infusion of hCRF. Slight increases in cortisol and ACTH levels were observed early during the infusion in some, but not all, of the patients at the lower doses. Modest increases in cortisol levels (2–3 × baseline) were seen in five out of nine patients at the highest doses, correlating with proportional increases in ACTH. These values had returned to baseline by the end of the infusion. Two patients had a decrease in cortisol/ACTH levels after infusion and two had no changes in cortisol levels despite increases in ACTH (including a patient known to have Addison's disease).

Correlations

Improvement in symptoms did not correlate with improvement in peritumoral edema by MRI assessment ($P = 0.32$); and with increment in serum cortisol levels at 60–90 min ($P = 0.28$), at 12 hours ($P = 0.55$), or at the end of hCRF infusion ($P = 0.90$). On univariate correlation analysis, no correlations were observed between changes in peritumoral edema by MRI (% change from baseline) and percentage changes from baseline in serum cortisol levels at 60–90 min ($r^2 = 0.011$), at 12 hours ($r^2 = 0.03$), or at the end of hCRF infusion ($r^2 = 0.10$).

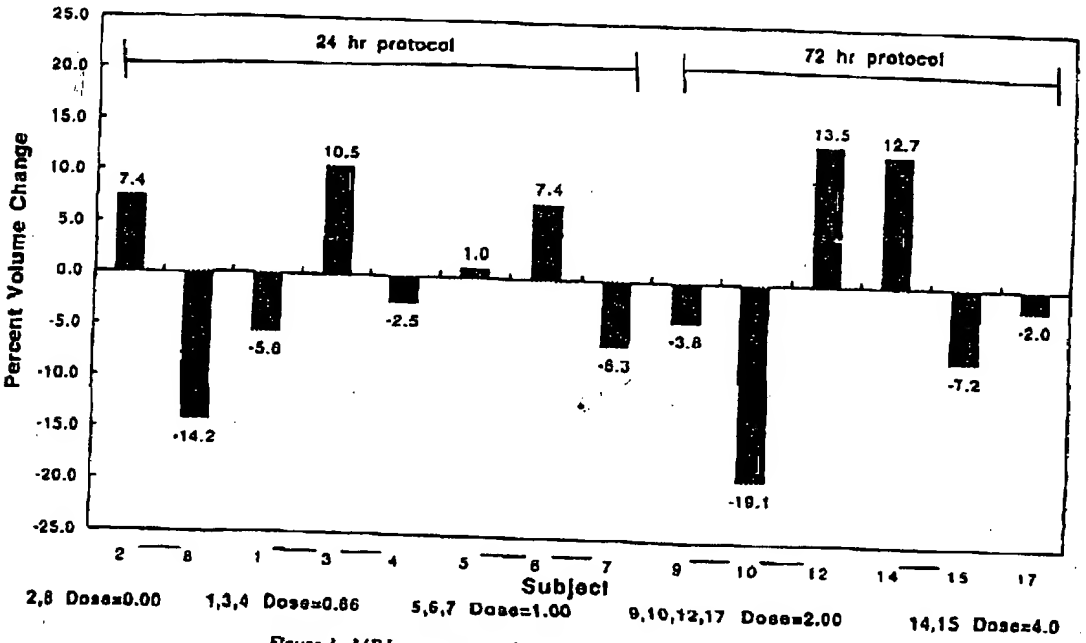

Figure 3. MRI assessment of change in peritumoral edema.

Table 4. Cortisol/ACTH levels during hCRF infusion.

No. of pts (dose) (mcg/kg/hr)	Cortisol[a] ACTH[b]	Level at baseline	Level at time points of hCRF infusion			
			90 min[c]	8 hrs	12 hrs	EOI
1 (0.66)	(C)	14.8	9.3	20.0	15.4	13.1
	(A)	–	–	–	–	–
2 (plac)	(C)	<2.5	<2.5	<2.5	<2.5	<2.5
	(A)	–	–	–	–	–
3 (0.66)	(C)	11.0	25.7	28.6	16.9	8.7
	(A)	44.0	22.0	43.0	13	6.0
4 (0.66)	(C)	<1.0	<1.0	<1.0	<1	<1.0
	(A)	<5.0	<5.0	6.0	<5	<5.0
5 (1.00)	(C)	9.3	12.2	14.4	12.5	15.0
	(A)	43.0	–	20.0	17.0	16.0
6 (1.00)	(C)	11.3	19.5	19.0	15.5	18.9
	(A)	13.0	34.0	26.0	17.0	24.0
7 (1.00)	(C)	15.8	14.2	17.2	16.9	16.4
	(A)	–	37.0	–	42.0	–
8 (plac)	(C)	9.4	0.8	24.0	10.3	19.4
	(A)	38.0	5.0	42.0	8.0	6.0
9 (2.00)	(C)	14.0	29.0	–	–	25.8
	(A)	<5.0	8.0	–	10.0	<5.0
10 (2.00)	(C)	28.6	17.9	–	30.4	17.9
	(A)	–	7.0	–	19.0	8.0
11 (2.00)	(C)	21.8	14.9	–	28.3	19.9
	(A)	58.0	53.0	–	<5.0	27.0
12 (2.00)	(C)	15.6	39.2	–	19.0	19.3
	(A)	–	39.0	–	19.0	9.0
13 (4.00)	(C)	10.3	22.1	–	–	–
	(A)	9.0	1.0	–	–	–
14 (4.00)	(C)	20.1	26.4	–	23.3	21.0
	(A)	22.0	74.0	–	32.0	65.0
15 (4.00)	(C)	9.6	27.4	–	27.6	11.8
	(A)	13.0	54.0	–	51.0	–
16 (4.00)	(C)	3.9	3.0	–	4.4	25.7
	(A)	58.0	938.0	–	640.0	207.0
17 (2.00)	(C)	4.4	9.7	–	16.6	9.8
	(A)	<5.0	16.0	–	11.0	<5.0

Abbreviation: EOI – end of infusion.
[a] Cortisol (C), (mcg/dl).
[b] ACTH (A), (pg/ml).
[c] Sixty minutes for patients 9–17.

Discussion

hCRF reduces water content in tumor and peritumoral tissue in brain tumor models *in vivo* when administered subcutaneously [9]. This effect appears not to depend on the release of adrenal steroids, but on a direct action on the tumor microvasculature. Our study showed that doses up to 1 μg/kg/h over 24 hours of this agent in humans were well-tolerated. At 2 μg/kg/h over 72 hours facial flushing is the predominant toxicity. No modification of the infusion is necessary for its resolution. Hypotension (grade 3) was dose-limiting in this study, at 4 μg/kg/h over 72 hours.

Improvement in signs and symptoms related to the peritumoral edema occurred in 10 patients and appeared at different times (median 24 hours) during the infusion of hCRF. None of those patients had received steroids for their brain metastasis. The small number of patients in this trial makes firm conclusions difficult, but no significant correlations were observed at the doses tested between symptomatic improvement and either MRI water content changes, or changes in serum cortisol.

It is not possible to compare doses in a per kg basis between rats and humans, so in the absence of measures of hCRF in serum, no estimations can be made of differences in hCRF dosing between these two species. Nevertheless, the MRI assessment failed to reveal changes as impressive as the ones reported in rats [9]. Low volume of tumor and edema of the patients in this study may have influenced the ability of the MRIs to detect changes that correlated with clinical improvement in some patients. Alternative hypotheses to be considered include: 1) the changes were short-lived and, thus, were not present when the reassessment occurred; 2) the beneficial effects seen with hCRF treatment were not related to a measurable reduction in peritumoral edema; or 3) the clinical improvement was not related to hCRF but to a placebo effect. Another important consideration is the timing used in this study for the assessment of changes in peritumoral edema. It was assumed that the optimal time to appreciate water contain changes was pre-infusion, and immediately before the end of the infusion. Since steady state levels are likely achieved earlier than the end of the infusion, it is possible that changes could have been appreciated during the infusion (e.g., at 12, 24, or 48 hours). In addition, intracranial events are sometimes detected later by neuroimaging procedures than when they actually occur, making the task of optimizing the timing of MRIs even more difficult.

The mechanism for hCRF-induced hypotension is not known. Co-administration of NaCl or pressors may possibly allow administration of doses ⩾4 μg/kg/h. However, administration of normal saline was associated in the preclinical studies with an increase in peritumoral edema [9]. Similarly, the use of pressors, while helping to support systemic blood pressure, could also potentially exert a negative influence in the resolution of edema. Therefore, advantages of dose intensity using the above approaches may not necessarily translate into a beneficial effect.

The improvement of symptoms seen in 10 patients in this trial is encouraging. The observation of clinical benefit in the absence of corticosteroids suggests hCRF may be an alternative to steroids for the treatment of patients with peritumoral brain edema. Exploration of different schedules or other routes of administration of this drug (e.g., subcutaneous), and efficacy studies with sufficient number of patients comparing the effect of this agent to the effect of traditional approaches for managing peritumoral brain edema, such as oral steroids, seem warranted.

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APPENDIX OF RELATED PROCEEDINGS

(No related matters)

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FROM

Deborah A. Rypacek (drypacek@fulbright.com)
PHONE NO. 713-651-5460

Should recipient confirm, by telephone, receipt of this facsimile transmission? No

COMMENTS:

Ms. Massard:

Per our telephone discussion, attached hereto is a copy of the October 15, 2004 Appeal Brief which contains Appendix X.

Please forward a revised Docketing Notice at your convenience.

Regards,

Charles P. Landrum (Deborah Rypacek - Secretary)